

WHAT IS CLAIMED IS:

1 1. A method of preparing a cell culture of propagating pancreatic cells
2 having the ability to be passed from one culture vessel to a second vessel at an initial
3 concentration of about 180 cells per square centimeter and expanded to about 1,800 cells per
4 square centimeter, while retaining the following properties: 90% PDX-1 positive and having
5 an insulin:actin mRNA ratio of between 1:100 and 1000:1, said method comprising the steps
6 of:

7 (a) isolating propagating pancreatic cells;

8 (b) transferring the cells to a culture medium containing growth hormone and
9 having 1% or less total volume of serum to selectively propagate cells having an insulin:actin
10 mRNA ratio of between 1:100 and 1000:1 and that are PDX-1 positive; and

11 (c) passaging the cells to provide a cell culture of propagating pancreatic cells
12 having the ability to be passed from one culture vessel to a second vessel at an initial
13 concentration of about 180 cells per square centimeter and expanded to about 1,800 cells per
14 square centimeter while retaining the following properties: 90% PDX-1 positive and having
15 an insulin:actin mRNA ratio of between 1:100 and 1000:1.

1 2. The method of claim 1, wherein the cells are maintained in a first
2 medium containing serum at between 1% and 4% of the total volume of the first medium
3 before transfer to a second medium containing growth hormone and 1% or less total volume
4 of serum.

1 3. The method of claim 1, wherein the cells are maintained in a first
2 medium containing serum at above 4% of the total volume of the first medium before transfer
3 to a second medium containing growth hormone and 1% or less total volume of serum.

1 4. The method of claim 3, wherein the cells are transferred from a first
2 medium containing serum at above 4% of the total volume of the serum to a second medium
3 containing growth hormone and less than 1% of total volume of serum by successive
4 transfers to media having successively lower amounts of serum.

1 5. The method of claim 1, wherein the isolated propagating pancreatic
2 cells have a mixture of PDX-1 positive and PDX-1 negative phenotypes.

1 6. The method of claim 1, wherein the cell culture has an insulin:actin
2 mRNA ratio of between 1:10 and 100:1.

1 7. A method of producing an aggregate of cultured pancreatic cells that
2 comprises an encapsulating mantle of ck-19 positive cells and an inner cell mass, wherein the
3 aggregate comprises 50-5000 pancreatic cells and has a diameter of between 50 and 300
4 microns, the method comprising the steps of

5 (a) culturing pancreatic cells on a substrate;

6 (b) removing the cells from the substrate;

7 (c) reseeding PDX-1 positive pancreatic cells on a substrate produced by step
8 (b); and

9 (d) culturing the cells of step (c) on the substrate of step (c) to provide an
10 aggregate of cultured pancreatic cells that comprise a surrounding mantle of ck-19 positive
11 cells and an inner cell mass, wherein the aggregate comprises 50-5000 pancreatic cells and
12 has a diameter of between 50 and 300 microns.

1 8. The method of claim 7, wherein the culturing of steps (a) or (c) takes
2 place in a medium containing growth hormone and less than 1% total volume of serum.

1 9. A method of providing pancreatic endocrine function to a mammal, the
2 method comprising the steps of

3 (a) producing an aggregate of cultured pancreatic cells by the method of step
4 7; and

5 (b) implanting the aggregate within the mammal.

1 10. A method of claim 9 where the cells are passage in media containing
2 between 1-2 mg per liter of recombinant growth hormone.

1 11. A method of claim 1 where the cells are passaged in media containing
2 recombinant human growth hormone.

1 12. A method of claim 1 where the cells are passaged in media containing
2 epithelial growth factor.

1 13. A culture of propagating pancreatic cells produced by the method of claim
2 1.

1 14. A culture of propagating pancreatic cells having the ability to be
2 passed from one culture vessel to a second vessel at an initial concentration of about 180 cells
3 per square centimeter and expanded to about 1,800 cells per square centimeter while retaining
4 the following properties: 90% PDX-1 positive and having an insulin:actin mRNA ratio of
5 between 1:100 and 1000:1.

1 15. An aggregate of pancreatic cells produced by the method of claim 7.

2 16. An aggregate of cultured pancreatic cells, comprising an encapsulating
3 mantle of CK19-positive cells and an inner cell mass, wherein the aggregate comprises 50-
4 5000 pancreatic cells and has a diameter of between 50 and 300 microns.